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Metabolic profiling of livers and blood from obese Zucker rats

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***Background/Aims*: Obesity frequently leads to changes in fatty acid metabolism with subsequent fatty infiltration in the liver.**

***Methods*: In this study, metabolic profile of the livers and blood from lean and obese Zucker rats was established based on quantitative nuclear magnetic resonance spectroscopy (NMR) analysis.**

***Results*: ^{1}H NMR on liver lipid extracts indicated significantly increased concentrations of total fatty acids and triglycerides. ^{31}P NMR on liver extracts revealed that obese livers have a compromised energy balance (low [ATP/ADP]) with decreased mitochondrial activity. Simultaneously, increased glycolytic activity was detected. The most pronounced differences were highly increased methionine and decreased betaine concentrations in obese animals. This suggests a significant alteration in methionine metabolism, which may be in part responsible for the development of steatosis, induction of mitochondrial dysfunction, and increased vulnerability of fatty livers to ischemia/reperfusion injury. A trend towards decreased hepatic glutathione concentrations as well as a reduced [PUFA/MUFA] ratio were present in the obese group, indicating increased oxidative stress and lipid peroxidation.**

***Conclusions*: In conclusion, NMR analysis on blood and liver tissue from obese Zucker rats reveals specific metabolic abnormalities in mitochondrial function and methionine metabolism, which result in a decreased hepatic energy state.**

***Keywords*: Nuclear magnetic resonance spectroscopy (NMR); Metabolomics; Methionine; Energy balance; Triglycerides; Obesity**

1. Introduction

Obesity frequently leads to changes in fatty acid metabolism with subsequent fatty infiltration of the liver and is referred to as nonalcoholic fatty liver disease (NAFLD). This pathophysiological entity comprises a wide spectrum of liver diseases that range from steatosis to non-alcoholic steatohepatitis (NASH) and ultimately to fibrosis and cirrhosis [1]. In the setting of obesity, insulin resistance is believed central to the development fatty livers [1,2]. Fatty infiltration of the liver can arise either from increased hepatic uptake or synthesis of fatty acids, or decreased fatty acid excretion or catabolism. A number of animal models are used to investigate hepatic metabolism in this setting. These include (i) targeted over-expression or naturally occurring mutations of genes that promote lipogenesis (e.g. SREBP-1), (ii) targeted deletion of genes that stimulate fatty acid oxidation (e.g. PPAR),

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Abbreviations: FFA, free fatty acids; NAFLD, nonalcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NMR, nuclear magnetic resonance spectroscopy; MAT, methionine adenosyltranserase; MUFA, mono-unsaturated fatty acids; PCA, perchloric acid; PUFA, poly-unsaturated fatty acids; SAM, S-adenosylmethionine; TCA, tricarboxylic acid cycle.

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(iii) high-carbohydrate, high-fat diets [3]. Each animal model demonstrates a distinct baseline metabolic profile, which needs to be taken into account when designing studies and interpreting results.

Obese Zucker rats are used widely for surgical research, to assess the efficacy of gastric bypass for achieving weight loss [4] and to investigate the influence of hepatic steatosis on liver injury during surgery or transplantation [5]. The latter is particularly important because fatty infiltration of the liver is recognized as a major risk factor for ischemia-reperfusion injury. Livers with significant fatty infiltration affect all aspects of liver transplantation including donors and recipients [6]. Moreover, some bariatric programs require liver biopsies as part of the risk assessment before gastric bypass surgery is recommended for morbid obesity [7].

To establish a meaningful animal model of obesity and hepatic steatosis, an accurate description of baseline metabolism is essential. A baseline profile is useful not only for judging the relevance of the model to human beings, but also for assessing the impact of subsequent experimental manipulations (e.g. surgery or ischemia-reperfusion).

The use of NMR-based metabolomics on tissue biopsies and body fluids allows for the assessment of dynamic changes in global metabolism and specifically for non-invasive blood markers which represents a novel and robust method of assessing organ response to pathophysiological stimuli [8–10]. It has been recently used to distinguish serum metabolic markers that predict coronary artery disease and hypertension [10]. In vivo MRS (magnetic resonance spectroscopy) has been performed on a limited basis in human beings with NASH [11,12]. However, most publications have reported the relative ratios of one metabolite to another, which, due to the complicated dynamic alterations in metabolism can often overlook significant changes. Quantification of obtained NMR based metabolites, as done by our group, will greatly facilitate comparison of data obtained in different experimental models [13,14].

Using NMR based quantitative metabolomics, it was our goal in the present study (i) to establish and validate NMR based metabolic differences between obese and lean Zucker rats, (ii) to establish concentration ranges for different metabolite markers that are highly specific for obesity; and most importantly (iii) to identify metabolic pathways which may explain the presumed increased susceptibility of livers from obese Zucker rats [15].

2. Material and methods

All animal experiments were carried out at the University of California at San Francisco with approval by the UCSF Committee on Animal Research. Animal care was in agreement with the National Institute of Health guidelines for ethical research (NIH publication No. 80-123, revised 1985).

2.1. Animal model

Obese Zucker rats lacking (homozygous recessive mutation (fa/fa) the leptin receptor were used. This lack results in increased food intake, insulin resistance and decreased energy expenditure (http://www.harlan.com/strain%20details/rats/zucker.html., accessed March 2005) Animals usually develop mild to moderate hepatic steatosis at approximately 8 weeks of life (personal communication with Harlan Laboratories, Indianapolis, IN, records on file).

Lean and obese Zucker rats (male, 10–12 weeks of age) were maintained on standard rodent chow (LabDiet 5008, PMI Nutrition International, Brentwood, MO). All rats were killed approximately between 8 and 10 am without a prior period of fasting. Rats were anesthetized with isoflurane (Abbott Lab, North Chicago, IL, USA) using a Summit rodent anesthesia machine (Summit Medical Equipment Co., OR, USA). The liver was exposed through a midline incision. Following mobilization of the liver, the abdominal aorta was catheterized with a 22 GA Angiocath (BD Infusion Therapy Inc., Sandy, UT, USA) and the liver subsequently removed and flushed free of blood. Tissue was sampled from the left lobe and snap-frozen for NMR analysis. In addition, blood was collected from the inferior vena cava at the time of animal sacrifice and stored at $-20\,^{\circ}C$.

2.2. Histology

Tissues were fixed in 10% formalin and then embedded in paraffin for light microscopy. Sections were cut at 5 μm and stained with hematoxylin and eosin (H&E) for histological examination at magnification 100× and 200×.

2.3. Liver and blood extraction

For NMR analysis, snap-frozen liver tissue (0.2 g) was subjected to acidic extraction (12% perchloric acid; PCA). Heparin-preserved whole blood (0.5 ml) was subjected to dual methanol/chloroform extraction and prepared as previously described [14].

2.4. Nuclear magnetic resonance spectroscopy on liver and blood extracts

All one- and two-dimensional ^{1}H MR spectra were obtained on a Bruker 500 MHz DRX spectrometer (Bruker Bisopin, Fremont, CA) using an inverse TXI probe. For metabolite identification in water-soluble and lipid extracts, a two-dimensional (2D)-H, C-HSQC (heteronuclear single quantum correlation) technique was used. The experiments were acquired with 512 increments and 256 scans per increment, using 90degrre pulse and a recovery delay of 1 s. The spectral width was 10 ppm in the proton dimension and 140 ppm in the ^{13}C dimension. Lactate (Lac3, CH_3) was used as a chemical shift reference for both carbon (21 ppm) and proton (1.32 ppm) axes. Tissue metabolites were identified based on the results from our chemical shift database. For metabolite quantification (proton), a standard water presaturation pulse program 'zgpr' was used to suppress water residue signal [10]. The total number of acquisitions was 40, the pulse delay of 12.8 s was applied between acquisitions for fully relaxed ^{1}H NMR spectra. An external standard, trimethylsilylpropionic acid (TMSP-d_4, 0.6 mmol/l for water-soluble and 1.2 mmol/l for lipid extracts) was used for metabolite quantification and as a chemical shift reference (0 ppm). Prior to ^{31}P NMR on liver water-soluble extracts, 100 mmol/l EDTA was added to the extracts for complexation of divalent ions (which results in decreased line broadening of ATP and ADP NMR signals). ^{31}P NMR was performed on a Bruker 300 MHz Avance system using a triple QNP probe. The operating frequency was 121.3 MHz, the total number of acquisitions was 6000, and WALTZ16 proton decoupling program was used. Methyldiphosphoric acid (MDP, 1.2 mmol/l) was used as an external standard for quantitation of metabolites in ^{31}P MR spectra and as a chemical shift reference (18 ppm). For post-processing spectral analysis and metabolite signal integration, the Bruker 1DWINNMR program was used.